

What is claimed is:

1. A method for identifying an MHC-binding peptide for an MHC monomer, or modified MHC monomer, said method comprising:
 - a) incubating under suitable liquid phase conditions a sample comprising:

at least one MHC monomer or modified MHC monomer having bound thereto a template MHC-binding peptide,

an excess amount of a first competitor peptide, and

a tracer MHC-binding peptide tagged with a detectable label

so as to allow competition between the first competitor peptide, the template peptide, and the tracer peptide for binding to the MHC monomer or modified MHC monomer, wherein the template peptide has lower or intermediate affinity as compared with the tracer peptide for the monomer; and
 - b) determining a difference in signal produced by the detectable label in the sample as compared with signal produced solely by monomer obtained from the sample after the incubation, wherein the difference indicates the first competitor peptide is an MHC-binding peptide for the monomer.
2. The method of claim 1, wherein the excess of the first competitor peptide is about 100-fold molar excess.
3. The method of claim 1, wherein the tracer peptide displaces at least 90% of the template peptide in a parallel competition assay conducted in the absence of the first competitor peptide.
4. The method of claim 1, wherein the suitable liquid phase conditions include incubating the sample for about 2 to 20 hours.
5. The method of claim 4, wherein the suitable liquid phase conditions further include incubating the sample at about 21 °C.

6. The method of claim 1, wherein the detectable label is a fluorophore.
7. The method of claim 6, wherein the monomer is attached to a solid support prior to determining the signal produced solely by the monomers in the sample.
8. The method of claim 7, wherein the MHC monomer or modified MHC monomer is biotinylated and the monomer is attached to the solid support via a biotin/avidin or streptavidin linkage.
9. The method of claim 6, wherein the fluorophore is fluorescein (FITC).
10. The method of claim 1, wherein the monomer is in a ternary complex further comprising beta-2 microglobulin.
11. The method of claim 1, wherein the difference is a decrease in the signal and binding of the first competitor peptide to the monomer is proportional to the amount of the decrease.
12. The method of claim 1, wherein the monomer is HLA class I.
13. The method of claim 12, wherein the monomer is HLA-A*020/Mart-1 26-35.
14. The method of claim 13, wherein the tracer peptide is HBc 18-27.
15. The method of claim 12, wherein the monomer is obtained from the sample in b) by cytometry.
16. The method of claim 1, wherein the method is repeated, except that a different competitor peptide is used.

17. The method of claim 1, wherein the determining comprises reading fluorescence using high throughput scanning.
18. The method of claim 1, wherein the monomer is HLA class I and monomer further binds with beta-2 microglobulin.
19. The method of claim 18, wherein the monomers is HLA subclass A, B or C.
20. The method of claim 18, wherein the first competitor peptide comprises from about 8 to about 12 amino acids.
21. The method of claim 20, wherein the monomer remains folded during the assay.
22. The method of claim 20, wherein the tracer peptide comprises from about 8 to about 12 amino acids and peptide exchange occurs without unfolding or denaturing of the monomer.
23. The method of claim 1, wherein the affinity of an exchanged competitor peptide is substantially equal to affinity of the first competitor peptide when folded into the binding pocket of the monomer during reconstitution of a ternary complex comprising the first competitor peptide and the monomer.
24. The method of claim 1, wherein the modified MHC monomer comprises cell surface domains of the MHC monomer but does not comprise other domains of the MHC monomer.
25. The method of claim 1, wherein the allele of the monomer is known and the determining indicates whether the first competitor peptide is specific for the allele of the monomer.

26. A method for measuring relative affinity of MHC-binding peptides for an MHC monomer, or modified MHC monomer, said method comprising:

- a) incubating under suitable liquid phase conditions a sample comprising:
 - at least one MHC monomer or modified MHC monomer having bound thereto a template MHC-binding peptide,
 - an excess amount of a first competitor peptide, and
 - a tracer MHC-binding peptide tagged with a detectable labelso as to allow competition between the first competitor peptide, the template peptide, and the tracer peptide for binding to the MHC monomer or modified MHC monomer, wherein the template peptide has lower affinity than the tracer peptide for the monomer; and wherein at least a portion of the first competitor peptide exchanges with the template peptide; and
- b) determining a difference in signal produced by the detectable label in the total sample as compared with signal produced solely by monomer obtained from the sample after the incubation, wherein the difference indicates affinity of the first competitor peptide for the monomer.

27. The method of claim 26, wherein the excess of the first competitor peptide is about 100-fold molar excess.

28. The method of claim 26, wherein the tracer peptide displaces at least 90% of the template peptide in a competition peptide exchange assay conducted in the absence of the first competitor peptide.

29. The method of claim 26, wherein the suitable liquid phase conditions include incubating the sample for about 2 to about 6 hours.

30. The method of claim 26, wherein the suitable liquid phase conditions further include incubating the sample at about 21 °C.

31. The method of claim 26, wherein the detectable label is a fluorophore.
32. The method of claim 31, wherein the monomer is attached to a solid support prior to determining the signal produced solely by the monomers in the sample.
33. The method of claim 32, wherein the MHC monomer or modified MHC monomer is biotinylated and the monomer is attached to the solid support via a biotin/avidin or streptavidin linkage.
34. The method of claim 31, wherein the fluorophore is fluorescein (FITC).
35. The method of claim 26, wherein the monomer is in ternary complex further comprises beta-2 microglobulin.
36. The method of claim 26, wherein the difference is a decrease in the signal and binding of the first competitor peptide to the monomer is proportional to the amount of the decrease.
37. The method of claim 26, wherein the monomer is HLA class I.
38. The method of claim 37, wherein the monomer is HLA-A*020/Mart-1 27-35.
39. The method of claim 26, further comprising obtaining the monomer from the sample in b) by cytometry.
40. The method of claim 26, wherein the method is repeated, except that a different competitor peptide is used.
41. The method of claim 40, wherein the tracer peptide is HBc 18-27.

42. The method of claim 26, wherein the determining comprises reading fluorescence using high throughput scanning.
43. The method of claim 26, wherein the monomer is HLA class I and monomer further binds with beta-2 microglobulin.
44. The method of claim 43, wherein the monomer is HLA subclass A, B or C.
45. The method of claim 43, wherein the first competitor peptide comprises from about 8 to about 12 amino acids.
46. The method of claim 45, wherein peptide exchange occurs without unfolding or denaturing of the monomer.
47. The method of claim 45, wherein the tracer peptide comprises from about 8 to about 12 amino acids and peptide exchange occurs without unfolding or denaturing of the monomer.
48. The method of claim 26, wherein the affinity of a exchanged competitor peptide is substantially the equal to affinity of the first competitor peptide when folded into the binding pocket of the monomer during reconstitution of a ternary complex comprising the first competitor peptide and the monomer.
49. The method of claim 26, wherein the modified MHC monomer comprises cell surface domains of the MHC monomer but does not comprise other domains of the MHC monomer.
50. The method of claim 26, wherein the allele of the monomers is known and the determining determines whether the first competitor peptide is specific for the allele of the monomer.

51. A method for measuring function of an MHC-monomer or modified MHC monomer bound to an exchanged peptide for staining a cell displaying a peptide-restricted T-cell receptor (TCR), said method comprising:

a) incubating under suitable liquid phase conditions a sample comprising:

MHC monomers or modified MHC monomers having

bound thereto a template MHC-binding peptide,

an excess amount of a first competitor peptide,

and

a tracer MHC-binding peptide tagged with a first detectable label

so as to allow competition between the first competitor peptide, the template peptide and the tracer peptide for binding to the MHC monomer or modified MHC monomer, wherein the template peptide has lower affinity than the tracer peptide for the monomer and wherein, in at least a portion of the monomers, the first competitor peptide exchanges with the template peptide to form exchanged monomers;

b) forming a multimer of the exchanged monomers obtained from a) by binding the exchanged monomers with a multivalent entity labeled with a second detectable label; and

c) determining binding of the exchanged monomers in the multimer with the TCR of the cell, wherein the binding indicates the first competitor peptide in the exchanged monomers is specific for the TCR.

52. The method of claim 51, wherein the MHC monomer or modified MHC monomer is biotinylated and the multivalent entity is streptavidin or avidin.

53. The method of claim 51, wherein the first detectable label is FITC and the second detectable label is PE.

54. The method of claim 51, wherein the excess is about 100-fold molar excess.

55. The method of claim 51, wherein the tracer peptide displaces at least 90% of the template peptide in a competition peptide exchange assay conducted in the absence of the first competitor peptide.

56. The method of claim 51, wherein the suitable liquid phase conditions include incubating the sample for about 6 to about 20 hours.

57. The method of claim 56, wherein the suitable liquid phase conditions further include incubating the sample at about 21 °C.

58. The method of claim 51, wherein the monomer is a ternary complex further comprising beta-2 microglobulin.

59. The method of claim 51, wherein the monomer is HLA class I.

60. The method of claim 59, wherein the monomer is HLA-A*020.

61. The method of claim 60, wherein the template peptide is Mart-1 26-35.

62. The method of claim 61, wherein the tracer peptide is HBc 18-27.

63. The method of claim 51, wherein the determining comprises reading fluorescence of the cells using high throughput scanning.

64. The method of claim 51, wherein the monomer is HLA class I and the monomer further comprises beta-2 microglobulin.

65. The method of claim 64, wherein the monomers is HLA subclass A, B or C.

66. The method of claim 51, wherein the first competitor peptide comprises from about 8 to about 12 amino acids.

67. The method of claim 66, wherein the tracer peptide comprises from about 8 to about 12 amino acids and peptide exchange occurs unfolding or denaturing of the monomer.

68. The method of claim 51, wherein peptide exchange occurs without unfolding or denaturing of the monomer.

69. The method of claim 51, wherein the binding of the multimer of exchanged monomers with the TCR is substantially equal to binding of a multimer prepared from the monomers in which the first competitor peptide is folded into the binding pocket of the monomer during reconstitution of a ternary complex.

70. The method of claim 51, wherein the modified MHC monomer comprises cell surface domains of the MHC monomer but does not comprise other domains of the MHC monomer.

71. The method of claim 51, wherein the peptide specificity of the TCR is known and the binding of the monomers in the multimer to the TCR indicates the exchanged monomer matches the peptide specificity of the TCR.

72. The method of claim 51, wherein the method is repeated except that a different competitor peptide is used in place of the first competitor peptide.

73. A system for identifying an MHC-binding peptide for an MHC monomer, or modified MHC monomer, said system comprising:

a) at least one MHC monomer or modified MHC monomer having bound thereto a template MHC-binding peptide,

b) a tracer MHC-binding peptide tagged with a detectable label

wherein the template peptide has lower affinity than the tracer peptide for the monomer, and

74. The system of claim 73, wherein the system further comprises an instruction for using the system.
75. The system of claim 73, wherein the monomer is HLA-A*020 and the template peptide is Mart-1 26-35.
76. The system of claim 73, wherein the tracer peptide is HBc 18-27.
77. The system of claim 73, wherein the detectable label is FITC.
78. The system of claim 73, wherein the monomer is biotinylated for attachment to an avidinated solid support.